

Gaussia GLOW-Juice BIG KIT (1 02 542)

Components include:

Gaussia GLOW-Juice	100 ml Buffer for measuring Gaussia-Luciferase – without substrate. Store at +4°C.
Coelenterazine(CTZ)	1 vial lyophilised substrate for 100 ml Gaussia GLOW-Juice (synth.) Coelenterazine for Renilla- and Gaussia- Luciferase Store at -80°C in the dark.
Reconstruction buffer	2 ml for dissolving the lyophilised Coelenterazine Store at -80°C in the dark.
2x Lysis-Juice 2	2 x 10ml dual concentrated Lysis-Buffer without detergents, for measurement of marine luciferases (Renilla/Gaussia) in mammalian cells. Store at +4°C.

Reconstruction:

This Gaussia GLOW-Juice BIG-KIT includes 5x 10 ml Test-Systems for Gaussia-Luciferase. Note: If the lyophilised Coelenterazine is dissolved in the Reconstruction buffer the stability will decrease after 30 Days. Please pipette the 2 ml Reconstruction buffer into the brown glass tube of lyophilised CTZ. It results a **50 x stock solution** that will be stable for at least **30 days** after reconstruction (**store at -80°C!!!**). The calculated amount of Coelenterazine stock solution has to be mixed into the measuring Gaussia GLOW-Juice **shortly before use** (2µl CTZ into 100 µl Gaussia GLOW-Juice).

The reagents should reach at least room temperature (20-25°C) before starting measuring the luciferases! Remainders of the mixed Gaussia GLOW-Juice should not be frozen again because it will lose noticeable activity.

Preparation of Cell Lysates:

Gaussia GLOW-Juice BIG-KIT includes Lysis-Juice 2. This buffer doesn't contain detergent, is dual concentrated and suitable for mammalian cells which were transfected with **Renilla/Gaussia/Firefly Luciferase**. Please dilute the dual concentrated lysis buffer with water or within your cell culture!

Standard protocol for Cells cultured in multiwell-plates

Required final volume of Lysis-Juice 2 per well:

Culture Plate	Vol. Lysis-Juice
6-well	500µl
12-well	250µl
24-well	100µl
48-well	65µl
96-well	20µl

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Standard protocol for Cells cultured in multiwell-plates

- Remove the growth medium from your adherent cells and wash the monolayer two or three times with phosphate buffered saline (PBS)
- Add the required volume of Lysis-Juice to each well (see Tabel)
- Place the plate on a shaker for 15 minutes at room temperature, additional up and down pipetting steps will increase the cell lysis. Freeze cells at -20 or -80°C and thaw them afterwards
- The cell lysate can be placed in storage tubes or measured in the plate by adding reconstructed Gaussia GLOW-Juice.

Standard Protocol

Program luminometer:

For the measurement we suggest a delay of 2 sec. after adding the reagent to the lysate and a measuring time of 5 sec..

- 1.) Transfer 20µl cell lysate into your luminometer tube.
- 2.) Add 100 µl **Gaussia GLOW-Juice**
- 3.) Start measurement

Standard procedure:

